

INTERNATIONAL SEARCH REPORT

Int. national application No.
PCT/BE 00/00081

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-9

A non-human genetically modified mammal comprising a mutation, a partial or total deletion in the genetic sequence encoding the wild type mammal alpha-fetoprotein (AFP) and pluripotential embryonic stem cell comprising said sequence encoding a mammal AFP.

2. Claim : 10

A study, testing and/or screening method and device of known or unknown molecules that are able to fix the alpha-fetoprotein or a portion thereof and that may be used as agonists or antagonists of oestrogens, for fertilization and/or contraceptive methods and compositions and/or the prevention and/or treatment of osteoporosis.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. Appl. Application No

PCT/BE 00/00081

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9622787 A	01-08-1996	US 5965528 A	12-10-1999
		AU 700975 B	14-01-1999
		AU 4903596 A	14-08-1996
		CA 2211324 A	01-08-1996
		EP 0805687 A	12-11-1997
		JP 10513347 T	22-12-1998
-----	-----	-----	-----
WO 0040693 A	13-07-2000	AU 2492400 A	24-07-2000
-----	-----	-----	-----
WO 0053759 A	14-09-2000	AU 3871200 A	28-09-2000
-----	-----	-----	-----

INTERNATIONAL SEARCH REPORT

International Application No

PCT/BE 00/00081

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/00 A01K67/027 C12N5/06 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A01K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, EMBASE, MEDLINE, CAB Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIN D.K. ET AL: ". alpha.- Fetoprotein gene sequences mediating Afr2 regulation during liver regeneration." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (21 JUL 1998) 95/15 (8767-8772).. XP000964629 the whole document	1-4
X	CIRILLO L.A. ET AL: "Developmental regulation of. alpha.- fetoprotein expression in intestinal epithelial cells of transgenic mice." DEVELOPMENTAL BIOLOGY, (1995) 168/2 (395-405).. XP000964762 the whole document	1-4

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 November 2000

Date of mailing of the international search report

13.02.01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hix, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/BE 00/00081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>TYNER A.L. ET AL: "The ontogeny of. alpha.- fetoprotein gene expression i the mouse gastrointestinal tract." JOURNAL OF CELL BIOLOGY, (1990) 110/4 (915-927)., XP000964763 the whole document</p>	1-4
X	<p>--- MILLONIG J H ET AL: "Molecular analysis of the distal enhancer of the mouse alpha - fetoprotein gene." MOLECULAR AND CELLULAR BIOLOGY, (1995 JUL) 15 (7) 3848-56., XP000964767 the whole document</p>	1-4
X	<p>--- H. CHEN ET AL.: "Identification of a cis-acting element in the rat alpha-fetoprotein gene and its specific binding proteins in F9 cells during retinoic acid-induced differentiation." JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 75, 1 January 1999 (1999-01-01), pages 25-34, XP000960630 the whole document</p>	9
Y	<p>--- BUTTERFIELD L.H. ET AL: "Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from. alpha.- fetoprotein." CANCER RESEARCH, (1 JUL 1999) 59/13 (3134-3142)., XP000960637 the whole document</p>	1-4
Y	<p>--- HENRIETTE M.-F. ET AL: "Negative regulation of the.alpha.-foetoprotein gene in fibroblasts: Identification and characterization of cis and trans elements." FOLIA BIOLOGICA, (1997) 43/1 (5-13)., XP000964925 the whole document</p>	1-4
Y	<p>YOUNG P R ET AL: "Construction and expression in vivo of an internally deleted mouse alpha - fetoprotein gene: presence of a transcribed Alu-like repeat within the first intervening sequence." NUCLEIC ACIDS RESEARCH, (1982 MAY 25) 10 (10) 3099-116., XP000964915 the whole document</p> <p style="text-align: center;">--- -/--</p>	1-4

INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/BE 00/00081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 22787 A (MURGITA ROBERT A) 1 August 1996 (1996-08-01) the whole document	1-9
E	WO 00 40693 A (LINDSAY STACE ;MULROY ROBERT (US); SEMENIUK DANIEL (US); ATLANTIC) 13 July 2000 (2000-07-13) page 20, paragraph 2	1-4
E	WO 00 53759 A (LINDSAY STACE ;MULROY ROBERT (US); SEMENIUK DANIEL (US); ATLANTIC) 14 September 2000 (2000-09-14) the whole document	1-4
T	SCOBY S. ET AL: "Identification of an enhancer and an alternative promoter in the first intron of the. alpha.- fetoprotein gene." NUCLEIC ACIDS RESEARCH, (1 OCT 2000) 28/19 (3743-3751)., XP000960628 the whole document	1-9

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 27 February 2001 (27.02.01)	
International application No. PCT/BE00/00081	Applicant's or agent's file reference P.ULB.52/WO
International filing date (day/month/year) 11 July 2000 (11.07.00)	Priority date (day/month/year) 12 July 1999 (12.07.99)
Applicant GABANT, Philippe et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
 07 February 2001 (07.02.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer S. Mafra Telephone No.: (41-22) 338.83.38
---	--

CLAIMS AS AMENDED IN THE PCT

5

CLAIMS

1. A non-human genetically modified mammal comprising a mutation, a partial or total deletion in the genetic sequence encoding the wild type mammal alpha-fetoprotein (AFP).

10

2. The non-human mammal according to claim 1, which is a mouse.

3. The non-human mammal according to claim 1, which comprises a heterozygous mutation, partial or total deletion in the genetic sequence encoding the wild type mammal alpha-fetoprotein (AFP).

15

4. The non-human mammal according to claim 1, which comprises an homozygous mutation, partial or total deletion in the genetic sequence encoding the wild type alpha-fetoprotein (AFP).

20

5. The non-human mammal according to claim 4, which is a sterile female.

6. The non-human mammal according to claim 4, which does not present a menstrual cyclization.

7. The non-human mammal according to claim 4, which does not allow an uteral nidification of an embryo.

25

8. Method for studying, testing and/or screening anti-osteoporosis fertilization and/or contraceptive methods, compounds and compositions by applying said method, compounds and composition upon the non-human, genetically modified mammal according to claim 1.

30

9. A pluripotential embryonic stem cell, preferably a mouse cell, comprising a partial or total deletion of a genetic sequence encoding a mammal alpha-fetoprotein (AFP).

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
18 January 2001 (18.01.2001)

PCT

(10) International Publication Number
WO 01/03501 A3

(51) International Patent Classification⁷: C12N 15/00,
A01K 67/027, C12N 5/06, 5/08

ROSCAM-SZPIRER, Josiane [BE/BE]; Avenue Jas-
sogne 27, B-1410 Waterloo (BE).

(21) International Application Number: PCT/BE00/00081

(74) Agents: VAN MALDEREN, Eric et al.; Office Van
Malderen, Place Reine Fabiola 6/1, B-1083 Brussels (BE).

(22) International Filing Date: 11 July 2000 (11.07.2000)

(81) Designated States (*national*): AU, CA, IL, JP, US.

(25) Filing Language: English

(84) Designated States (*regional*): European patent (AT, BE,
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC,
NL, PT, SE).

(26) Publication Language: English

(30) Priority Data:

60/143,269

12 July 1999 (12.07.1999) US

Published:

— With international search report.

(71) Applicant (*for all designated States except US*): UNIVER-
SITE LIBRE DE BRUXELLES [BE/BE]; Avenue F.D.
Roosevelt 50, CP 161, B-1050 Brussels (BE).

(88) Date of publication of the international search report:
12 July 2001

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): GABANT, Philippe
[BE/BE]; Rue Van Bommel 9, B-1210 Brussels (BE).

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*



WO 01/03501 A3

(54) Title: NON-HUMAN GENETICALLY MODIFIED MAMMAL LACKING THE ALPHA-FETOPROTEIN

(57) Abstract: The present invention is related to a non-human genetically modified mammal comprising a mutation, a partial or total deletion of the genetic sequence encoding the wild type mammal alpha-fetoprotein (AFP).

INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/BE 00/00081

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/00 A01K67/027 C12N5/06 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A01K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, EMBASE, MEDLINE, CAB Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIN D.K. ET AL: ". alpha.- Fetoprotein gene sequences mediating Afr2 regulation during liver regeneration." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (21 JUL 1998) 95/15 (8767-8772).. XP000964629 the whole document	1-4
X	CIRILLO L.A. ET AL: "Developmental regulation of. alpha.- fetoprotein expression in intestinal epithelial cells of transgenic mice." DEVELOPMENTAL BIOLOGY, (1995) 168/2 (395-405).. XP000964762 the whole document	1-4

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 November 2000

Date of mailing of the international search report

13. 02. 01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hix, R

INTERNATIONAL SEARCH REPORT

Internat. Application No

PCT/BE 00/00081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>TYNER A.L. ET AL: "The ontogeny of. alpha.- fetoprotein gene expression i the mouse gastrointestinal tract." JOURNAL OF CELL BIOLOGY, (1990) 110/4 (915-927)., XP000964763 the whole document</p>	1-4
X	<p>--- MILLONIG J H ET AL: "Molecular analysis of the distal enhancer of the mouse alpha - fetoprotein gene." MOLECULAR AND CELLULAR BIOLOGY, (1995 JUL) 15 (7) 3848-56., XP000964767 the whole document</p>	1-4
X	<p>--- H. CHEN ET AL.: "Identification of a cis-acting element in the rat alpha-fetoprotein gene and its specific binding proteins in F9 cells during retinoic acid-induced differentiation." JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 75, 1 January 1999 (1999-01-01), pages 25-34, XP000960630 the whole document</p>	9
Y	<p>--- BUTTERFIELD L.H. ET AL: "Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from. alpha.- fetoprotein." CANCER RESEARCH, (1 JUL 1999) 59/13 (3134-3142)., XP000960637 the whole document</p>	1-4
Y	<p>--- HENRIETTE M.-F. ET AL: "Negative regulation of the.alpha.-foetoprotein gene in fibroblasts: Identification and characterization of cis and trans elements." FOLIA BIOLOGICA, (1997) 43/1 (5-13)., XP000964925 the whole document</p>	1-4
Y	<p>YOUNG P R ET AL: "Construction and expression in vivo of an internally deleted mouse alpha - fetoprotein gene: presence of a transcribed Alu-like repeat within the first intervening sequence." NUCLEIC ACIDS RESEARCH, (1982 MAY 25) 10 (10) 3099-116., XP000964915 the whole document</p>	1-4
	<p>---</p> <p>-/--</p>	

INTERNATIONAL SEARCH REPORT

Inter- national Application No

PCT/BE 00/00081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 22787 A (MURGITA ROBERT A) 1 August 1996 (1996-08-01) the whole document ---	1-9
E	WO 00 40693 A (LINDSAY STACE ;MULROY ROBERT (US); SEMENIUK DANIEL (US); ATLANTIC) 13 July 2000 (2000-07-13) page 20, paragraph 2 ---	1-4
E	WO 00 53759 A (LINDSAY STACE ;MULROY ROBERT (US); SEMENIUK DANIEL (US); ATLANTIC) 14 September 2000 (2000-09-14) the whole document ---	1-4
T	SCOHY S. ET AL: "Identification of an enhancer and an alternative promoter in the first intron of the. alpha.- fetoprotein gene." NUCLEIC ACIDS RESEARCH, (1 OCT 2000) 28/19 (3743-3751).. XP000960628 the whole document -----	1-9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BE 00/00081

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-9

A non-human genetically modified mammal comprising a mutation, a partial or total deletion in the genetic sequence encoding the wild type mammal alpha-fetoprotein (AFP) and pluripotential embryonic stem cell comprising said sequence encoding a mammal AFP.

2. Claim : 10

A study, testing and/or screening method and device of known or unknown molecules that are able to fix the alpha-fetoprotein or a portion thereof and that may be used as agonists or antagonists of oestrogens, for fertilization and/or contraceptive methods and compositions and/or the prevention and/or treatment of osteoporosis.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter. Appl. Application No

PCT/BE 00/00081

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9622787	A	01-08-1996	US 5965528 A	12-10-1999
			AU 700975 B	14-01-1999
			AU 4903596 A	14-08-1996
			CA 2211324 A	01-08-1996
			EP 0805687 A	12-11-1997
			JP 10513347 T	22-12-1998

WO 0040693	A	13-07-2000	AU 2492400 A	24-07-2000

WO 0053759	A	14-09-2000	AU 3871200 A	28-09-2000


PCT

WFO

PC

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P.ULB.52/WO		FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/BE00/00081	International filing date (day/month/year) 11/07/2000	Priority date (day/month/year) 12/07/1999	
International Patent Classification (IPC) or national classification and IPC A01K67/027			
Applicant UNIVERSITE LIBRE DE BRUXELLES et al.			
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 8 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 1 sheets.</p>			
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input checked="" type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input checked="" type="checkbox"/> Certain documents cited VII <input checked="" type="checkbox"/> Certain defects in the international application VIII <input checked="" type="checkbox"/> Certain observations on the international application 			
Date of submission of the demand 07/02/2001		Date of completion of this report 28.09.2001	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016		Authorized officer Hix, R Telephone No. +31 70 340 3898	



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/BE00/00081

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-15 as originally filed

Claims, No.:

1-9 as received on 10/08/2001 with letter of 30/07/2001

Drawings, sheets:

1/5-5/5 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/BE00/00081

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
☐ paid additional fees.
☐ paid additional fees under protest.
☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☒ complied with.
☐ not complied with for the following reasons:

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☐ all parts.
☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5-8
	No:	Claims	1-4, 9
Inventive step (IS)	Yes:	Claims	5-8
	No:	Claims	1-4, 9

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/BE00/00081

Industrial applicability (IA) Yes: Claims 1-9
 No: Claims

2. Citations and explanations
 see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

Re Item IV

Lack of unity of invention

Claim 10 has been deleted, thereby establishing a unifying concept for claims 1 to 9 according to Rule 13.1 PCT.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

NOVELTY (Art. 33(2) PCT)

1.} D1 and D2 both involve the construction of transgenic mice in which part of the alpha-fetoprotein (AFP) gene has been deleted. D1 involves the generation of mice which comprise an active natural AFP-gene and the mice in D2 still comprise a complete functional AFP gene.

1.1} However, due to the extremely broad formulation of the independent claim 1, where claim 1 does not specify the phenotype of the transgenic mice, but only that the modified mammal comprises a "mutation, a partial or total deletion in the genetic sequence encoding the wild type mammal alpha-fetoprotein", claim 1 is considered to also encompass transgenic mice which comprise a mutation in the AFP gene and yet still exhibit the phenotype of a functional AFP.

1.2} The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1 to 4 is not new in respect of prior art D1 and D2 as defined in the regulations (Rule 64(1)-(3) PCT).

2.} Similarly independent claim 9 simply defines the pluripotent embryonic stem cell as comprising "a partial or total deletion of a genetic sequence encoding a mammal alpha-fetoprotein".

2.1} D5 describes a CAT reporter plasmid containing the rat AFP 5'-regulatory region (-7040 to +7) adjacent to the CAT gene (pAFPCAT) which was stably transfected into mouse F9 cells, pluripotent stem cells of teratocarcinoma. Page 27, right column, last

paragraph, states that in order to determine which cis-acting elements confer retinoic acid (RA) mediated activation of the AFP gene in differentiating F9 cells, a series of deletion mutants of pAFPCAT were stably transfected into F9 cells. The 5' end of the AFP gene was removed up to -2.6kb and a deletion of the nucleotide sequence between -2611 to -1855 resulted in a five-fold drop in CAT activity.

2.2} Therefore the IPEA is of the opinion that D5 anticipates the subject-matter of claim 9. The subject-matter of claim 9 is therefore not novel in the light of the disclosures of D5 according to Article 33{2} PCT.

INVENTIVE STEP (Art. 33(3) PCT)

3.} Document D1 is considered to represent the most relevant state of the art and discloses AFP gene sequences mediating Afr2 regulation during liver regeneration.

3.1} The subject-matter of claims 5 to 8 differs in that the presence of the mutated, partial or totally deleted AFP gene sequence in the non-human genetically modified mammal results in a sterile female and/or may be used in the screening or testing of anti-osteoporosis fertilization and/or as a contraceptive.

3.2} The problem to be solved by the subject matter of claims 6 to 8 may therefore be regarded as the provision of an alternative use for a non-human genetically modified mammal comprising a mutated, partial or totally deleted AFP gene sequence.

3.3} The solution that the presence of the mutated, partial or totally deleted AFP gene sequence in the non-human genetically modified mammal results in a sterile female and/or may be used in the screening or testing of anti-osteoporosis fertilization and/or as a contraceptive has not been suggested in any of the available prior art documents. The link between AFP and female reproduction and fertility is not disclosed in the state of the art.

3.4} In the light of the above reasoning, the subject-matter of claims 5 to 8 is considered to involve an inventive step according to Article 33{3} PCT.

Re Item VI

Certain documents cited

WO-A-0 040 693 {Atlantic Biopharmaceuticals, Inc.} published on 13.7.2000 , filed on 6.1.2000 and claiming priority from 6.1.99.

* Although WO-A-0 040 693 {Atlantic Biopharmaceuticals, Inc.} does not constitute prior art within the meaning of Rule 64.1(b), it appears to disclose all the features of claims 1 to 4. It might therefore be taken into consideration in the regional phase before the EPO. No check has been made as to whether the priority of this application has been validly claimed.

The priority documents pertaining to the present application were not available at the time of establishing this first written opinion. Hence, it is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the above document (cf page) cited in the international search report could become relevant to assess whether the claimed subject matter satisfy the criteria set forth in Art. 33(1) PCT.

Re Item VII

Certain defects in the international application

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in documents D1 to D5 are not mentioned in the description, nor are these documents identified therein.

Re Item VIII

Certain observations on the international application

- 1 The application does not meet the requirements of Article 6 PCT because claims are not clear for the following reasons:
 - 1.1 Claims 6 and 7 define the non-human mammal in terms of negative features of the result to be achieved, such as; "characterized in that it does not present a menstrual cyclization" or "does not allow an uteral...". For the sake of the clarity of said claims it is preferable to use positive technical features of the non-human modified mammal rather than negative functional features.
- 2 Claims 6 and 7 therefore do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined. The claims attempts to define the subject-matter in terms of the result to be achieved which merely amounts to a statement of the underlying problem. The technical features necessary for achieving this result are however missing.
- 3 An independent claim must specify clearly all the essential technical features necessary to define the invention. In the present case the particular mutation or partial deletion in the nucleic acid sequence sequence encoding AFP which results in the loss of sterility or menstrual cycle is considered to be essential technical features which allow the unambiguous characterization of the non-human transgenic mammal and the method and use of the present application. Accordingly the location and the technical details of the particular mutation or partial deletion in the nucleic acid sequence sequence encoding AFP which results in the loss of sterility or menstrual cycle must be incorporated into the independent claims in order to satisfy the requirements of clarity according to Article 6 and Rule 6{3}{b} PCT.

CLAIMS

1. A non-human genetically modified mammal comprising a mutation, a partial or total deletion in the genetic sequence encoding the wild type mammal
5 alpha-fetoprotein (AFP).

2. The non-human mammal according to claim 1, characterized in that it is a mouse.

3. The non-human mammal according to claim 1 or 2, characterized in that it comprises a heterozygous
10 mutation, partial or total deletion in the genetic sequence encoding the wild type mammal alpha-fetoprotein (AFP).

4. The non-human mammal according to claim 1 or 2, characterized in that it comprises an homozygous mutation, partial or total deletion in the genetic sequence
15 encoding the a wild type alpha-fetoprotein (AFP).

5. The non-human mammal according to claim 4, characterized in that it is a sterile female.

6. The non-human mammal according to claim 5; characterized in that it does not present a menstrual
20 cyclization.

7. The non-human mammal according to claim 5, characterized in that it does not allow an uteral nidification of an embryo.

8. Use of the non-human mammal according to
25 any one of the preceding claims, for the study, the testing and/or the screening of anti-osteoporosis fertilization and/or contraceptive methods, compounds and compositions.

9. A pluripotential embryonic stem cell, preferably a mouse cell, comprising a partial or total
30 deletion of a genetic sequence encoding a mammal alpha-fetoprotein (AFP).

10. A study, testing and/or screening method and device of known or unknown molecules that are able to fix the alpha-fetoprotein or a portion thereof and that may

be used as agonists or antagonists of oestrogens, for fertilization and/or contraceptive methods and compositions and/or the prevention and/or the treatment of osteoporosis.

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P. ULB. 52/WO	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/BE 00/ 00081	International filing date (day/month/year) 11/07/2000	(Earliest) Priority Date (day/month/year) 12/07/1999
Applicant UNIVERSITE LIBRE DE BRUXELLES et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/BE 00/00081

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-9

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-9

A non-human genetically modified mammal comprising a mutation, a partial or total deletion in the genetic sequence encoding the wild type mammal alpha-fetoprotein (AFP) and pluripotential embryonic stem cell comprising said sequence encoding a mammal AFP.

2. Claim : 10

A study, testing and/or screening method and device of known or unknown molecules that are able to fix the alpha-fetoprotein or a portion thereof and that may be used as agonists or antagonists of oestrogens, for fertilization and/or contraceptive methods and compositions and/or the prevention and/or treatment of osteoporosis.

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/00 A01K67/027 C12N5/06 C12N5/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A01K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, EMBASE, MEDLINE, CAB Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JIN D.K. ET AL: ". alpha.- Fetoprotein gene sequences mediating Afr2 regulation during liver regeneration." PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (21 JUL 1998) 95/15 (8767-8772).. XP000964629 the whole document	1-4
X	CIRILLO L.A. ET AL: "Developmental regulation of. alpha.- fetoprotein expression in intestinal epithelial cells of transgenic mice." DEVELOPMENTAL BIOLOGY, (1995) 168/2 (395-405).. XP000964762 the whole document	1-4

-/--

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"E" earlier document but published on or after the international filing date

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"O" document referring to an oral disclosure, use, exhibition or other means

"G" document member of the same patent family

"P" document published prior to the international filing date but later than the priority date claimed

Date of the actual completion of the international search

Date of mailing of the international search report

30 November 2000

13.02.01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hix, R

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>TYNER A.L. ET AL: "The ontogeny of. alpha.- fetoprotein gene expression i the mouse gastrointestinal tract." JOURNAL OF CELL BIOLOGY, (1990) 110/4 (915-927)., XP000964763 the whole document</p>	1-4
X	<p>--- MILLONIG J H ET AL: "Molecular analysis of the distal enhancer of the mouse alpha - fetoprotein gene." MOLECULAR AND CELLULAR BIOLOGY, (1995 JUL) 15 (7) 3848-56., XP000964767 the whole document</p>	1-4
X	<p>--- H. CHEN ET AL.: "Identification of a cis-acting element in the rat alpha-fetoprotein gene and its specific binding proteins in F9 cells during retinoic acid-induced differentiation." JOURNAL OF CELLULAR BIOCHEMISTRY, vol. 75, 1 January 1999 (1999-01-01), pages 25-34, XP000960630 the whole document</p>	9
Y	<p>--- BUTTERFIELD L.H. ET AL: "Generation of human T-cell responses to an HLA-A2.1-restricted peptide epitope derived from. alpha.- fetoprotein." CANCER RESEARCH, (1 JUL 1999) 59/13 (3134-3142)., XP000960637 the whole document</p>	1-4
Y	<p>--- HENRIETTE M.-F. ET AL: "Negative regulation of the.alpha.-fetoprotein gene in fibroblasts: Identification and characterization of cis and trans elements." FOLIA BIOLOGICA, (1997) 43/1 (5-13)., XP000964925 the whole document</p>	1-4
Y	<p>--- YOUNG P R ET AL: "Construction and expression in vivo of an internally deleted mouse alpha - fetoprotein gene: presence of a transcribed Alu-like repeat within the first intervening sequence." NUCLEIC ACIDS RESEARCH, (1982 MAY 25) 10 (10) 3099-116., XP000964915 the whole document</p> <p>---</p> <p>-/--</p>	1-4

INTERNATIONAL SEARCH REPORT

International Application No.

PCT 00/00081

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96 22787 A (MURGITA ROBERT A) 1 August 1996 (1996-08-01) the whole document ---	1-9
E	WO 00 40693 A (LINDSAY STACE ;MULROY ROBERT (US); SEMENIUK DANIEL (US); ATLANTIC) 13 July 2000 (2000-07-13) page 20, paragraph 2 ---	1-4
E	WO 00 53759 A (LINDSAY STACE ;MULROY ROBERT (US); SEMENIUK DANIEL (US); ATLANTIC) 14 September 2000 (2000-09-14) the whole document ---	1-4
T	SCOHY S. ET AL: "Identification of an enhancer and an alternative promoter in the first intron of the. alpha.- fetoprotein gene." NUCLEIC ACIDS RESEARCH, (1 OCT 2000) 28/19 (3743-3751)., XP000960628 the whole document -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/JP 00/00081

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9622787	A	01-08-1996	US 5965528 A	12-10-1999
			AU 700975 B	14-01-1999
			AU 4903596 A	14-08-1996
			CA 2211324 A	01-08-1996
			EP 0805687 A	12-11-1997
			JP 10513347 T	22-12-1998
WO 0040693	A	13-07-2000	AU 2492400 A	24-07-2000
WO 0053759	A	14-09-2000	AU 3871200 A	28-09-2000